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**(54) TESTING DEVICE**

(57) The present invention relates to a sheet-like test device characterized in that the portions other than the portions that function as liquid-absorptive sections of a liquid-absorptive sheet is subjected to a hydrophobicity treatment so that a plurality of liquid-absorptive portions for causing a reaction with a test liquid are formed separately from one another. With the test device of the invention, multiple items of tests can be performed conveniently and expeditiously. The device can be mass-produced in an industrially advantageous manner through a hydrophobicity treatment employing, for example, printing.

## Description

## TECHNICAL FIELD

5 The present invention relates to a test device which, as compared to a conventional microplate, makes chemical or microbiological tests that require multiple reaction items simpler and more expeditious, and which can be mass-produced in an industrially advantageous manner.

## BACKGROUND ART

10 A chemical test involving a step of causing a chemical sample to react with a certain reagent, or a microbiological test involving a step of incubating a microorganism in a sample—especially a test that requires treatment of numerous samples, reaction with various reagents, incubation in many systems, or like procedures—generally employs a plastic microplate provided with a plurality of wells functioning as reaction containers or culture vessels.

15 In a test employing a microplate of this type, reactions or incubations of a microorganism are independently performed in each well of the plate. For example, in MIC measurement performed by Microbroth dilution method using a microplate, antimicrobial chemicals having different concentrations are added to a plurality of wells, in which the growth of the microorganism of interest is observed so as to thereby determine the Minimum inhibitory concentration.

20 As test devices usable for such a test, there are commercially sold microplates of a type prepared, for example, by dispensing a predetermined amount of an antimicrobial chemical to each well of the microplate and subsequently drying or freezing for storage. However, these test devices unavoidably come to be expensive, since the cost of a microplate itself is considerably high. Therefore, there exists an increased demand for low-price devices.

Another problem is that, when numerous tests are conducted through use of a single sample, a predetermined amount of sample must be placed dropwise in each well, making operations intricate.

25 In view of the foregoing, an object of the present invention is to provide a test device which can be mass-produced at reduced cost, and, *inter alia*, which permits simple and expeditious chemical or microbiological tests for multiple reaction items.

## DISCLOSURE OF THE INVENTION

30 Under the above-mentioned circumstances, the present inventors conducted careful studies and found that, through use of a liquid-absorptive sheet in which the portions other than the portions corresponding to wells (liquid-absorptive sections) of a microplate have undergone hydrophobicity treatment, leakage of test liquid from the liquid-absorptive sections is prevented, the amount of water absorbed by the water absorptive sections is made constant, and  
35 such microplates can be mass-produced at reduced cost because hydrophobicity treatment such as printing is employed for production. They also found that when the thus-prepared liquid-absorptive sections are used in combination with a diffusion section for supplying a test liquid, dropwise addition of a test liquid to a single point of the diffusion section enables testing of multiple items. The present invention was achieved based on these findings.

40 Accordingly, the present invention provides a sheet-like test device characterized in that the portions other than the portions that function as liquid-absorptive sections of a liquid-absorptive sheet are subjected to a hydrophobicity treatment so that a plurality of water-absorptive portions which are caused to react with a test liquid are formed separately from one another (hereinafter referred to as a "sheet-like test device of the present invention").

45 The present invention also provides a test device characterized by comprising the aforementioned sheet-like test device of the present invention and a diffusion section which maintains contact with at least a portion of each liquid-absorptive section and supplies a test liquid to the liquid-absorptive section (hereinafter referred to as a "test device of the present invention").

## BRIEF DESCRIPTION OF THE DRAWINGS

50 Fig. 1 shows a test device of the present invention manufactured in accordance with Example 2, wherein numeral 1 indicates liquid-absorptive sections, numeral 2 indicates a diffusion section, and numeral 3 indicates a test-liquid supply hole; and  
Fig. 2 shows a test device of the present invention (including its housing).

## 55 BEST MODES FOR CARRYING OUT THE INVENTION

Examples of material for the liquid-absorptive sheet of the sheet-like test device of the present invention include rayon fibers, polyester fibers, acrylic fibers, Vinyon fibers, cotton fibers, glass fibers, and other fibers made of a syn-

thetic or natural material. Of these, acrylic non-woven fabric is preferred in view of its high liquid-absorptive characteristics. The liquid-absorptive sheet preferably has a liquid absorption value of 0.020 g/cm<sup>2</sup> or more, preferably 0.08-0.2 g/cm<sup>2</sup>.

The liquid absorption value of the present invention is obtained in accordance with the following procedure, which is a modification of a method of Crow *et al.* (INDA-TEC, 207 (1991)).

- 1) A piece of sponge (made of polyurethane, 8.5 cm × 14 cm, thickness 3 cm) which has been caused to sufficiently absorb water in advance is immersed in a plate containing purified water;
- 2) A test piece (1 cm × 1 cm) is placed on the water-soaked sponge and another piece of sponge having the same size as the first sponge is placed on the test piece;
- 3) The plate carrying the sandwiched test piece is placed together with the plate in a humid box, allowed to stand at room temperature (20-25°) for five hours, and then the weight of the sample is measured; and
- 4) The weight of the sample before absorbing water is subtracted from the weight of the sample after absorbing water, to thereby determine the liquid absorption value.

The liquid-absorptive sheet used in the present invention preferably has a thickness of 300-3000 μm, particularly preferably 500-2000 μm. When the thickness is less than 300 μm, a test liquid is not sufficiently retained in liquid-absorptive sections, and therefore, accurate measurement cannot be performed due to insufficient reaction. In contrast, when the thickness is in excess of 3000 μm, simplicity of the below-mentioned hydrophobicity treatment will be lost.

The sheet-like test device of the present invention is obtained through hydrophobicity treatment of the portions other than the portions that function as liquid-absorptive sections so that a plurality of liquid-absorptive sections are formed separately from one another.

The hydrophobicity treatment is preferably conducted by use of a print method; i.e., by allowing permeation of a liquid containing as a predominant component a hydrophobic resin into predetermined portions (other than liquid-absorptive sections) of a liquid-absorptive sheet and drying. Examples of the hydrophobic resins used in the present invention include polyurethane resins, polyacrylic resins, polyester resins, polyamide resins, polyvinyl chloride resins, polystyrene resins, fluororesins, and other rubber-modified products. Any of known resins that are conventionally used, such as those employed as ink binders, may be used so long as their water-solubility and moisture-absorptivity are not excessively high. Solvents which may be used to dissolve or disperse these hydrophobic resins are selected from among a variety of organic solvents in accordance with the hydrophobic resin employed, and examples thereof include toluene or cyclohexanone. To the liquid containing a hydrophobic resin (hydrophobic ink) as a predominant component there may be added, according to needs, additives such as a colorant; an inorganic or organic filler; a water repellent such as a fluororesin or silicone; a surfactant; or a viscosity-modifier.

In order to cause the hydrophobic ink to permeate into the predetermined portions of a liquid-absorptive sheet, typical silk screen printing is preferably performed. Briefly, a hydrophobic ink is preferably applied onto the predetermined portions of a liquid-absorptive sheet for permeation and is subsequently subjected to drying. The hydrophobic ink may be applied once, or preferably several times, in order to ensure hydrophobicity treatment.

In order to cause the thus-applied hydrophobic ink to sufficiently permeate into the liquid-absorptive sheet so as to reach the backside thereof, any the following methods is preferably performed: (1) a method in which a pressure is applied to the ink-application surface of the sheet, (2) a method in which a suction force is applied to the surface opposite the ink-application surface of the sheet, (3) a method in which air is blown on the side of the ink-application surface of the sheet, or (4) a suitable combination of two or more methods selected from among (1), (2), and (3).

No particular limitation is imposed on the method (1); i.e., application of pressure to the ink-application surface of the sheet. For example, roll-pressing is preferred.

In this method, at least two rolls, e.g., a metal roll having a mirror-polished surface and a rubber roll, are used in combination and disposed one on the other. A hydrophobic-ink-coated, liquid-absorptive sheet is inserted therebetween so that the ink-application surface contacts the metal roll. A pressure is continuously applied between the two rolls while they are rotated.

In this case, the mirror-polished metal roll may be heated. In the case in which the hydrophobic ink is not completely dried, application of pressure alone may result in permeation of the hydrophobic ink to the backside of the liquid-absorptive sheet without additional heating.

When this method is used, no special metallic die of a specific shape is needed, and the sheet may be easily pressed with excellent productivity through regulation of the rotational speed of the rolls and the distance between the rolls or nip pressure.

Upon application of pressure, preferably a film which does not absorb a hydrophobic ink is placed on the ink-application surface of the liquid-absorptive sheet and an absorptive material such as paper is preferably placed on the surface opposite the ink-application surface of the sheet. By this method, excessive ink that penetrates the sheet onto the backside thereof under pressure can be absorbed by the absorptive material, to thereby prevent the ink from adhering

to the rolls or overflowing on the liquid-absorptive sheet.

As an alternative method for the application of a pressure onto the ink-application surface of the liquid-absorptive sheet, there may be employed a planar pressing method, wherein an unheated or heated sheet which is flat in its entirety or a sheet patterned in accordance with the ink-application pattern of the hydrophobic ink is used.

When the planar pressing method is used, if the ink applied onto the liquid-absorptive sheet is not completely dried, the pressurizing plate may be at ambient temperature as in the case of the above-described roll-pressing method. However, when pressure is applied after the ink has dried, the pressurizing plate must be heated so as to soften or melt the hydrophobic ink.

When pressure is applied by use of a patterned pressurizing plate, needless to say, the pressurizing plate and the ink-application pattern of the liquid-absorptive sheet must be pressed after being positioned accurately.

In this case also, upon application of pressure, if the ink applied onto the liquid-absorptive sheet is not completely dried, preferably a film which does not absorb a hydrophobic ink is placed on the ink-application surface of the liquid-absorptive sheet and an absorptive material such as paper is placed on the surface opposite the ink-application surface of the sheet.

In the case in which pressure is applied through use of a heated planar plate after the applied ink has dried, preferably the pressurizing planar plate and the pressure-receiving surface are both subjected to mold-releasing treatment of heat resistance so that the softened or molten hydrophobic ink does not adhere to the pressurizing planar plate and the pressure-receiving surface.

When the method (2); i.e., application of suction force to the surface opposite the ink-application surface of the sheet, is used in association with ink application means adapted to the silk screen printing process, a vacuum-suction device provided for securing the sheet for printing to the printer itself may be used as is, and if necessary, through adjustment of the diameters of the suction holes or their arrangement pattern, suction force may be applied to ink simultaneously with printing to thereby cause the ink to permeate the sheet. When the printer has no vacuum suction device, a separate but similar suction device may be provided, with which suction force is applied to ink which is not yet dry after printing to thereby permeate the sheet with ink.

In the case of method (3); i.e., a method in which air is blown on the side of the ink-application surface of the sheet to thereby apply pressure for permeation of the ink, ink may be caused to permeate into the sheet by placing the ink-applied, liquid-absorptive sheet on a wire-net-like receiving base and by applying air pressure onto the ink-application surface of the sheet by use of a blower equipped with a hood.

Furthermore, chambers of existing air-pressure/vacuum molding machines for forming plastic sheets may also be utilized. In this case, a separately provided receiving base in the form of a wire net, instead of a female die for molding, is attached to the inside of the chamber, and a liquid-absorptive sheet to which ink has been applied is placed on the receiving base. Subsequently, the chamber is closed and air is discharged without the application of vacuum. Pressure is applied onto the ink-application surface by the feeding of compressed air, to thereby achieve permeation of the ink.

When the combination of (2) and (3); i.e., feeding air on the ink-application side and suction of air on the opposite side, is employed, there may be used chambers of existing air-pressure/vacuum molding machines described above in connection with method (3). In this case also, a wire net receiving base, which is used instead of a female die for molding, is attached to the inside of the chamber, and a liquid-absorptive sheet to which ink has been applied is placed on the receiving base, followed by closure of the chamber and simultaneous pressurization by application of compressed air onto the ink-application surface and suction by use of vacuum from the opposite side.

In typical air-pressure/vacuum molding machines, a heating apparatus for heating plastic sheets and a molding apparatus are disposed in line. However, since the above-described methods (2) and (3) require no heating, a pressurized-air or vacuum apparatus alone, which is manufactured independently, may be connected downstream of a silk screen printer which may be followed by an optional drying apparatus if necessary, to thereby sequentially perform application of ink, permeation of the ink, and drying of the ink.

No particular limitation is imposed on the shapes and sizes of liquid-absorptive sections remaining after hydrophobicity treatment is performed, and they may be selected according to the amount of a test liquid and the degree of liquid absorption. Generally, preferable liquid-absorptive sections are circular or square having, e.g., a diameter or side length of approximately 0.2-1 cm. In this case, the amount of the test liquid which is caused to be absorbed by a single liquid-absorptive section is approximately 0.025-0.2 ml in general.

No particular limitation is imposed on the number of such liquid-absorptive sections, and it may arbitrarily be determined through the volume of a test liquid, the number of test items, etc.

In order to regulate the volume of liquid absorbed by the liquid-absorptive sections, a material fixed with a water-absorptive polymer may be used. Examples of the water-absorptive polymers include soluble starch; mannan; agar; sodium alginate; plant-derived polysaccharide (such as Arabic gum); microbial polysaccharide (such as dextran, xanthan gum, or Gellan gum); cellulose substances (such as methylcellulose, carboxymethylcellulose, or other species of cellulose); polyvinyl alcohol; a polyacrylic acid sodium salt; and graft copolymers thereof. Examples of techniques for fixation of these water-absorptive polymers onto the liquid-absorptive sections include dipping a printed liquid-absorptive

tive sheet in a liquid obtained by dissolving a water-absorptive polymer in water, an organic solvent, etc. and drying; and adding the above water-absorptive polymer liquid dropwise to liquid-absorptive sections and drying.

The test device of the present invention includes the aforementioned sheet-like test device and a diffusion section which maintains contact with at least a portion of each liquid-absorptive section and supplies a test liquid to the liquid-absorptive section. The ratio of the liquid absorption value in the liquid-absorptive sections to that of the diffusion section (absorption/diffusion) is 2 or more, preferably 5-15. When the ratio is less than 2, diffusion of the test liquid into the diffusion section for causing the absorptive sections to absorb the test liquid may become difficult.

Example materials of the diffusion section are the same as the materials listed for the liquid-absorptive sheet, and cotton non-woven fabric is preferred in view of enhanced diffusion characteristics and low liquid absorption.

Of these materials, preferred ones are those having a liquid absorption value of 0.005-0.05 g/cm<sup>2</sup>, particularly 0.008-0.02 g/cm<sup>2</sup>, and a liquid diffusion rate, as determined by time necessary for diffusion of water for an entirety of a rectangular sheet (1 cm × 10 cm) when its shorter side is dipped in purified water, of 15 minutes or less, particularly preferably 30 seconds to 3 minutes.

The diffusion section used in the present invention is provided to supply a test liquid and diffuse it to the liquid-absorptive sections. No particular limitation is imposed on the shape, size, and thickness of the diffusion section, and they may be selected according to the liquid absorption value of the test liquid applied to the liquid-absorptive sections, the number of the liquid-absorptive sections, etc. In general, the shape of the diffusion section is preferably sheet-like, and those having a thickness of approximately 0.05-0.6 mm are preferably used.

The liquid-absorptive sections of the above-described sheet-like test device are arranged so as to contact the diffusion section at least partially.

No particular limitation is imposed on the method of fixation of the liquid-absorptive sections of the sheet-like test device to the diffusion section. For example, there may be employed a method in which liquid-absorptive sections and part of the diffusion section are bonded by use of an adhesive (e.g., a hydrophilic adhesive or a water-soluble polymer); a method in which the liquid-absorptive sections disposed on the diffusion section are partially bonded by (heat) pressing; a method in which the diffusion section and the liquid-absorptive sections are joined by seaming; and a method in which the liquid-absorptive sections are inserted into cuts provided in the diffusion section.

A more preferable embodiment of the test device of the present invention is a sheet-like test device having a plurality of liquid-absorptive sections, and in addition, at the central location of a plurality of the liquid-absorptive sections, a test-liquid supply hole, and also having a diffusion section made of a liquid-diffusive sheet, wherein the sheet-like test device is arranged such that the test-liquid supply hole and a plurality of liquid-absorptive sections are brought into communication with one another on the diffusion section (See Fig. 1).

Further, the test device of the present invention is preferably distributed as accommodated in a waterproof casing. In a particularly preferable embodiment for the accommodation of the device in a waterproof casing there are provided a sheet-like test device having a waterproof bottom part and a cover having holes corresponding to a plurality of liquid-absorptive sections and a test liquid-supply hole to possess a plurality of liquid-absorptive sections and a test liquid-supply hole, wherein the diffusion section is a liquid-diffusive sheet, and the sheet-like test device arranged such that the test-liquid supply hole and a plurality of liquid-absorptive sections are brought into communication with one another on the diffusion section is secured to the casing (See Fig. 2). Furthermore, the cover preferably has an additional outer cover for distribution.

Examples of the water-resistant materials used as the bottom part and the cover include plastic sheets made of polyethylene, polypropylene, polystyrene, etc.; and glass.

To conduct a test through use of the sheet-like test device of the present invention, a test liquid is added dropwise in a specific amount to each liquid-absorptive section. The amount of the test liquid is determined according to the area and liquid absorption value of the liquid-absorptive sections.

To conduct a test by use of the test device of the present invention, test liquid may be poured in a specific amount to only one spot of the diffusion section, and the test liquid thereby diffuses throughout the diffusion section and is absorbed by a plurality of liquid-absorptive sections. In the case of the above-described preferable embodiment, test liquid may be added dropwise to the liquid-supply holes provided in the liquid-absorptive sections. The amount of the test liquid is determined in accordance with the area, liquid absorption value, etc. of the diffusion section and the liquid-absorptive sections.

In the present invention, test liquid absorbed by each of the liquid-absorptive sections reacts with a variety of reactive reagents. The reactive reagents may be contained in a test device in advance or added after absorption of the liquid. They are preferably contained in advance so that tests may be performed quickly, so long as they do not raise problems in respect to, for example, stability. No particular limitation is imposed on the reactive reagents used in the present invention, and examples include reagents for measurement of enzyme activity such as coloring substrates and fluorescent substrates; reagents for identification of microorganisms, such as sugar, amino acids, and fatty acids; reagents for measurement of drug susceptibility of microorganisms against antibiotics, etc.; and test reagents for antibacterial agents.

These reactive reagents may be fixed through addition of a solution of the reagent to pre-sterilized liquid-absorptive sections, and drying under reduced pressure. In this case, the above-described water-absorptive polymer may be added to the reactive reagent.

In the above-described manner, reactive reagents of different species and different concentrations can be fixed onto a plurality of liquid-absorptive sections of the test device of the present invention. Therefore, multiple items of reactions can be tested by a single trial. In this case, the reactive reagent, e.g., L-alanine-p-nitroanilide or L-proline- $\beta$ -naphthylamide, which is a coloring substrate used for measurement of enzyme activity in a test liquid (such as suspension of bacteria, body fluid, cerebrospinal fluid, or emulsified foodstuff), is preferably added in an amount of 0.01-0.5 wt.% based on the amount of the test liquid absorbed in the liquid-absorptive sections. When a fluorescent substrate such as 4-methylumbelliferyl- $\beta$ -D-galactopyranoside or L-alanine-7-amido-4-methylcoumarin is used as a reagent, a preferable amount of the reagent is 0.001-0.02 wt.% based on the amount of the test liquid absorbed in the liquid-absorptive sections.

When sugar, amino acids, fatty acids, or their intermediate metabolites are used as reagents for identification of microorganisms, their amounts are preferably 0.1-5 wt.% based on the amount of the test liquid absorbed in liquid-absorptive sections. When antibiotics are used as reagents for measurement of drug susceptibility of microorganisms, or when antibacterial agents are used for an antibacterial activity test, their concentrations may be arbitrarily determined in accordance with the target drugs and microorganisms of interest.

Moreover, gelling agents may also be added to liquid-absorptive sections in order to increase the viscosity of an absorbed test liquid to thereby obtain sufficient gel strength at the liquid-absorptive sections. Examples of the gelling agents include natural polymers such as xanthan gum, carageenan, Gelan gum, Arabic gum, locust bean gum, guar gum, tragacanth gum, or crystalline cellulose; and synthetic polymers such as polyvinylpyrrolidone, polyvinyl alcohol, polyethylene glycol, polypropylene glycol, polyacrylamide, polyacrylic acid, hydroxyethyl cellulose, or hydroxypropyl cellulose.

These gelling agents may be fixed onto the liquid-absorptive sections in a manner similar to that employed for fixation of the above-described reagents. In this case, they are preferably added in an amount of 0.1-1 wt.% (natural polymer) or 0.05-0.5 wt.% (synthetic polymer) based on the amount of the test liquid absorbed by the liquid-absorptive sections.

Furthermore, nutrients such as peptone may be added with an aim of culturing bacteria contained in the test liquid, and cations such as alkali metal salts and alkaline earth metal salts may be added to solutions of reagents so as to increase the gel strength and reaction sensitivity; thus, these components may be incorporated in the liquid-absorptive sections.

In addition, other nutrients, surfactants, and cations such as alkali metal salts may be added to test liquids.

## EXAMPLES

The present invention will next be described in more detail by way of examples. However, the present invention should not be construed as being limited to thereto.

### Example 1

The entire surface of a liquid-absorptive piece made of acrylic nonwoven fabric (A-200; Kuraray Trading Co., Ltd.) having a thickness of 1000  $\mu$ m was screen-printed with an ink shown in Table 1 below and by a print method also shown in Table 1 below, to thereby obtain a test device having non-printed circular portions of 7.5 mm diameter. Test on Sealing Effect by Printing with a Hydrophilic Ink;

The outer periphery (12 mm x 12 mm) of each of the non-printed portions was cut to make samples for measuring the liquid-absorption value.

With regard to the control sample, a piece (10 mm x 10 mm) was cut out from a non-printed liquid absorptive piece, and the liquid-absorption value was measured.

The measurement was performed in accordance with the method of Crow *et al.* For the control samples, data were processed as follows: (Measured amount of liquid absorption) x {(Surface area of 7.5mm  $\varnothing$ ) / (Area of 10mm x 10mm)}.

The results are shown in Table 1.

Table 1

No.	Print method	Ink type	Liquid absorption (g/absorptive sections)	Ratio
1	F2B1	Acrylic	0.105	210%
2	F3	Acrylic	0.056	112%
3	F3	Acryl + F	0.049	98%
4	F2B1	Acryl + F	0.051	102%
5	F3	Acryl + Si	0.043	86%
6	F2B1	Acryl + Si	0.050	100%
7	F1B1	Acryl + Si	0.070	140%
8	F1 roll press	Acryl + F	0.050	100%
Control	-	-	0.050	100%

\*Control;  $0.114\text{g/cm}^2 \times \text{Area of } 7.5\text{mm } \varnothing = 0.050\text{g}$

#### Ink type

Acrylic resin ..... Sericol 13 medium (Teikoku Ink Co., Ltd.)  
 Solvent ..... Sericol G-002 solvent (Teikoku Ink Co., Ltd.)  
 Fluorine ..... Comb-shaped polymer LFB-4015 (Soken Kagaku K.K.)  
 Silicone ..... Comb-shaped polymer LSI-60 (Soken Kagaku K.K.)

#### Mixing ratio

Acrylic	Acrylic resin	80 parts by weight
	Solvent	20 parts by weight
Acryl + Fluorine	Acrylic resin	80 parts by weight
	Solvent	10 parts by weight
	Fluorine	10 parts by weight
Acryl + Silicon	Acrylic resin	85 parts by weight
	Solvent	15 parts by weight
	Silicon	10 parts by weight

#### Print Method

Plate ... Number of lines: 70 mesh,

Thickness of resist: 90  $\mu\text{m}$

Printing (by a known screen print method)

F2B1: Printing was performed consecutively twice from the front face, and subsequent to drying, once from the back face.

F2 to F4: Consecutively twice to four times from the front face.

F1B1: Once from the front face, drying, and once from the back face.

## Roll Press Method

After printing, each liquid-absorptive piece, with its printed face upward, was sandwiched between an OPP film placed on the upper side and a wiper placed on the lower side and roll-pressed, to thereby cause the ink to penetrate.

As a result, preferable print methods could be selected through measurement of the liquid absorption value. Print methods identified as Nos. 2, 3, 4, 5, 6, and 8 in Table 1 were found to be excellent methods.

## Example 2

Liquid-absorptive pieces used in the test: With the exception of twelve 7.5 mm $\varnothing$  non-printed portions, the entirety of the surface of an acrylic nonwoven fabric (A-200 sheet) having a liquid absorption value of 0.114 g/cm<sup>2</sup> was subjected to uniform printing (print method No. 11). Subsequently, the printed piece was punched to provide a 106 mm x 38 mm rectangular piece, and a 13 mm x 18 mm elliptic hole was formed in its central portion by punching (Fig. 1). Development layer: Through use of an adhesive (Copoly 5404D, Nippon Synthetic Chemical Industry Co., Ltd.) a PET film (thickness; 75  $\mu$ m) was fixed to one side of a cotton nonwoven fabric (PS-140, Asahi Chemical Industry Co., Ltd.) having a liquid absorption value of 0.008 g/cm<sup>2</sup>, and the fabric was then processed into a 15 mm x 110 mm piece by punching.

Liquid-impermeable base plate and cover: The base plate has dimensions that permit accommodation of the above-mentioned liquid-absorptive piece for testing and development layer. The cover has openings corresponding to the positions of the non-printed portions and the punched central portion. Manufacture method: On the base plate were placed the development layer, the control liquid-absorptive piece, and the cover, in this order. Then, the control liquid-absorptive piece and the development layer were secured in place by the base plate placed above and the cover placed below. In order to ensure fixture, the base plate and the cover were fixed to each other with adhesive.

The development layer of the thus-obtained test device is partially exposed at punched-through portions of the liquid-absorptive piece. Therefore, when the test liquid is added dropwise to such exposed portion(s), the test liquid will reach the liquid-absorptive sections through the development layer (Fig. 2).

## Test Example 2

Through use of the test device obtained in Example 2, tests were performed according to the following test method. Also, comparative tests were performed by the microplate method. The results are shown in Tables 2 and 3.

## Test method:

Bacterial strain provided for the test:

E.coli ATCC 11775, K.pneumoniae ATCC 13883

## Reaction items and preparation method:

1. ALA : L-alanine-p-nitroanilide (product of SIGMA, 6mM pH 7.0),
2. PNP : p-nitrophenyl- $\beta$ -D-galactopyranoside (product of SIGMA, 6mM pH 7.0),
3. PYR : Pyroglutamyl- $\beta$ -naphthylamide (product of SIGMA, 3mM pH 7.0),
4. GLR : p-nitrophenyl- $\beta$ -D-glucuronide (product of SIGMA, 6mM pH 7.0),
5. PHE : p-nitro-L-phenylalanine (product of SIGMA, 4mM pH 7.3),
6. PRO : Proline- $\beta$ -naphthylamide (product of SIGMA, 3mM pH 7.0),
7. IND : Tryptophan (product of Wako Pure Chemical Industries, Ltd., 0.1% pH 7.4),
8. BGL : p-nitrophenyl- $\beta$ -D-glucopyranoside (product of SIGMA, 6mM pH 6.3),
9. NGL : p-nitrophenyl-N-acetyl- $\beta$ -D-glucosaminide (product of SIGMA, 2mM pH 7.0),
10. NIT : KNO<sub>3</sub> (product of Wako Pure Chemical Industries, Ltd., 0.5% pH 7.0)
11. BIP : 5-Bromo-3-indolyl phosphate (product of Wako Pure Chemical Industries, Ltd., 4mM pH 8.0)
12. XYL : p-nitrophenyl- $\beta$ -xyloside (product of SIGMA, 6mM pH 7.0)

For each of the above-mentioned items, a solution was prepared to have the final substrate concentration shown in corresponding parenthesis, and contain 0.5% peptose peptone (manufactured by DIFCO), 1% casein peptone (manufactured by MARCOR Development Corp.), and 0.1% xanthan gum (manufactured by San-Eigen F.F.I. Inc.). Each substrate solution was sterilized and dispensed directly to a test device in an amount of 50  $\mu$ l x number of 7.5 mm $\varnothing$  non-printed circular portions of the test device, and dried under reduced pressure.



Also, for control, each substrate solution was added dropwise to the wells of a microplate in an amount of 100  $\mu$ l per well, and immobilized likewise.

Culture test method and method for judgment:

Fresh bacteria were cultured in a sheep blood agar culture media-N "Nissui" (Nissui Pharmaceutical Co., Ltd.) for one day (18 to 24 hours), and suspended in a 0.1% gellan gum (manufactured by MERCK) solution to make the turbidity equivalent to that of a standard nephelometry liquid, McFarland #0.5. The bacterium suspension (1 ml) was supplied into each test device through the supply hole open at the central portion thereof. In order to maintain the sealing, a cover was put on the test device. The bacterium suspension immediately diffused and was absorbed in the circular non-printed sections within 6 minutes. The amount of absorbed test liquid was 50  $\mu$ l  $\pm$  10  $\mu$ l.

To each well of the control microplate, the bacterium suspension was added dropwise in an amount of 100  $\mu$ l per well through use of a pipette. After incubation at 37°C for 4 hours, positive(+)/negative(-) was visually determined from the color developed in each substrate. For test item Nos. 3, 6, 7, and 10, detection reagents (3, 6: a cinnamaldehyde reagent (bioMerieux Vitek Inc.), 7: Kovac's reagent (Nissui Pharmaceutical Co., Ltd.), 10: nitrite detection reagent I and nitrite detection reagent II (Nissui Pharmaceutical Co., Ltd.)) were added dropwise in an amount of 25 to 50  $\mu$ l, and positive(+)/negative(-) was determined after a lapse of 5 to 10 minutes.

Table 2

### Results;

#### E. coli ATCC 11775

	ALA	PNP	PYR	IND	BGL	NGL
	GLR	PHE	PRO	NIT	BIP	XYL
Microplate method	+	+	-	+	-	-
Method of the invention	+	+	-	+	-	-

Table 3

#### K. pneumoniae ATCC 13883

	ALA	PNP	PYR	IND	BGL	NGL
	GLR	PHE	PRO	NIT	BIP	XYL
Microplate method	+	+	+	-	+	-
Method of the invention	+	+	+	-	+	-

As is apparent from the above tables, the method using the test device of the present invention showed results identical to those shown by the microplate method. Therefore, through use of the test device of the present invention in which a predetermined amount of test liquid is poured into a single supply hole, can be performed simply and expeditiously a test involving multiple reaction items. Also, when the test device is overturned, the test liquid is not spattered since the test liquid is sealed up in the liquid-absorptive sections, thereby more effectively securing safety in terms of biohazard as compared to microplate-type devices.

### INDUSTRIAL APPLICABILITY:

The test device of the present invention can be mass-produced in an industrial advantageous manner, since it can

be manufactured through hydrophobicity treatment such as printing using a hydrophobic ink. Further, if the test device is additionally provided with a diffusion section, there is no need to dispense a test liquid to each well as in the case of conventional microplates. This is because the test liquid is poured into a single site of the diffusion section to be absorbed by each liquid-absorptive section, so that a test is conducted simply and expeditiously. Moreover, a test is conducted more expeditiously because a reagent or the like needed for the test can be contained in the absorptive sections in advance.

# Claims

1. A sheet-like test device characterized in that the portions other than the portions that function as liquid-absorptive sections of a liquid-absorptive sheet is subjected to a hydrophobicity treatment so that a plurality of liquid-absorptive portions for causing a reaction with a test liquid are formed separately from one another.
2. The sheet-like test device according to claim 1, wherein the thickness of the liquid-absorptive sheet is 300-3000  $\mu\text{m}$ .
3. The sheet-like test device according to claim 1 or 2, wherein the hydrophobicity treatment is carried out by causing a liquid containing as a predominant component a hydrophobic resin to permeate into predetermined portions of the liquid-absorptive sheet and drying.
4. A test device characterized by comprising a sheet-like test device as described in claim 1 and a diffusion section which maintains contact with at least a portion of each liquid-absorptive section and supplies a test liquid to the liquid-absorptive section.
5. The test device according to claim 4, wherein the thickness of the liquid-absorptive sheet is 300-3000  $\mu\text{m}$ .
6. The test device according to claim 4 or 5, wherein the hydrophobicity treatment is carried out by causing a liquid containing as a predominant component a hydrophobic resin to permeate into predetermined portions of the liquid-absorptive sheet and drying.
7. The test device according to any one of claims 4 through 6, wherein the ratio of the liquid absorption value in the liquid-absorptive sections to that of the diffusion section (absorption/diffusion) is 2 or more.
8. The test device according to any one of claims 4 through 7, wherein the sheet-like test device has a plurality of liquid-absorptive sections and a test-liquid supply hole, the diffusion section is a liquid-diffusive sheet, and the sheet-like test device is arranged such that the test-liquid supply hole and a plurality of the liquid-absorptive sections are brought into communication with one another on the diffusion section.
9. A test device set which comprises a test device as described in claim 8, a waterproof bottom part, and a cover having holes corresponding to a test liquid-supply hole and a plurality of liquid-absorptive sections of a sheet-like test device, wherein the test device is fixed onto or accommodated within the combination of the waterproof bottom part and the cover.

Fig. 1

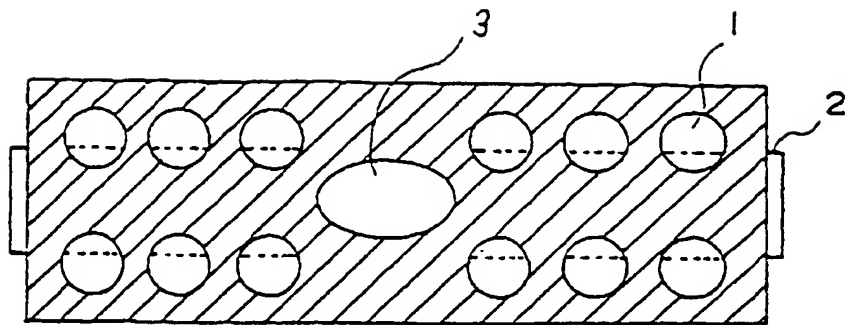
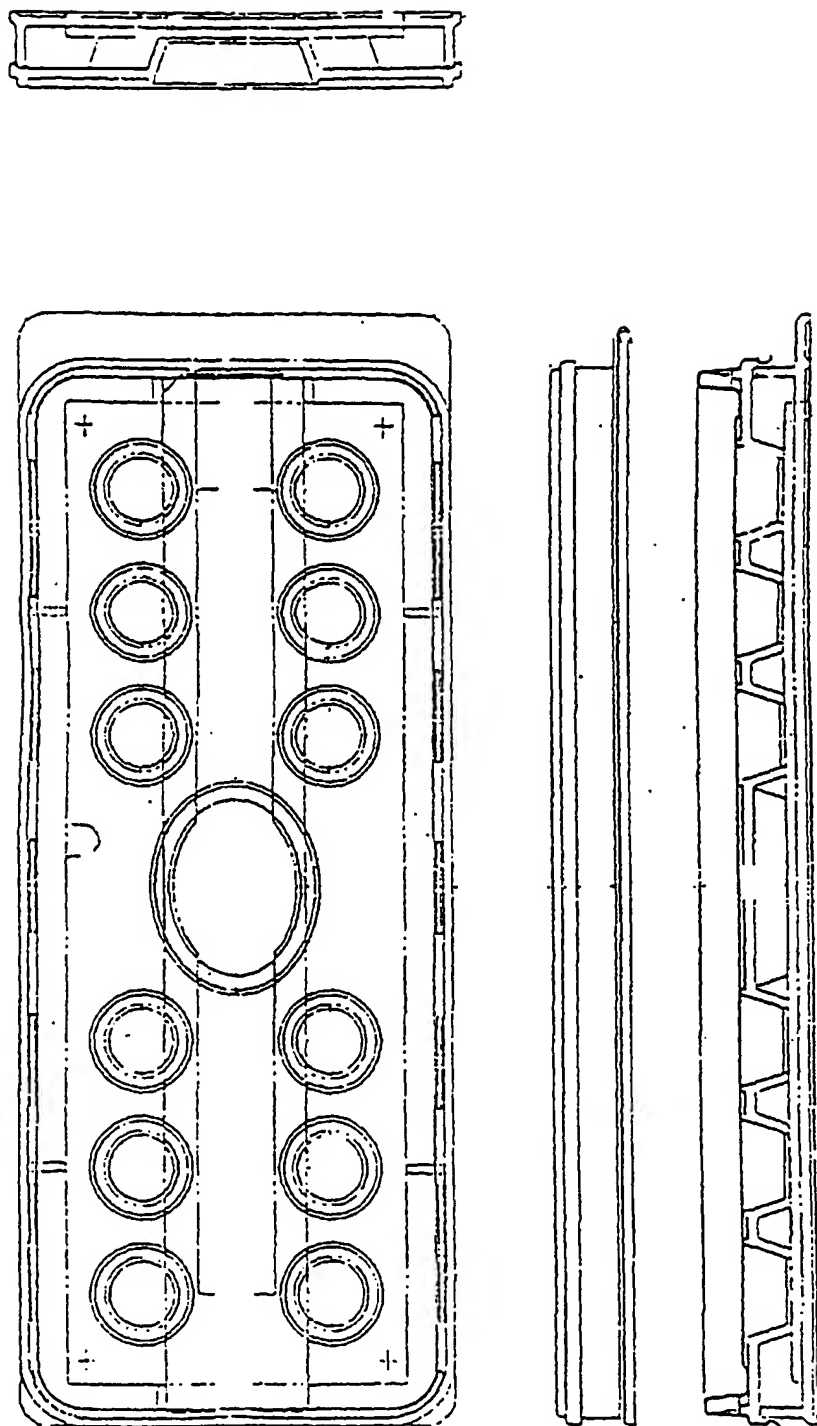


Fig. 2



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/04020

## A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl<sup>6</sup> C12M1/00, G01N1/28, G01N21/78, G01N31/22, G01N33/52

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int. Cl<sup>6</sup> C12M1/00, G01N1/28, G01N21/78, G01N31/22, G01N33/52

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Jitsuyo Shinan Koho	1926 - 1997	Jitsuyo Shinan Toroku
Kokai Jitsuyo Shinan Koho	1971 - 1997	Koho
Toroku Jitsuyo Shinan Koho	1994 - 1997	1996 - 1997

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP, 4-148699, A (Hitachi, Ltd.), May 21, 1992 (21. 05. 92), - Claims; page 2, lower right column, lines 1 to 6; page 4, upper left column, line 18 to upper right column, line 3 (Family: none)	1 - 3
Y		4 - 7
Y	JP, 8-166379, A (Nissui Pharmaceutical Co., Ltd.), June 25, 1996 (25. 06. 96), Abstract (Family: none)	4 - 7
A	Microfilm of the specification and drawings annexed to the request of Japanese Utility Model Application No. 136752/1987 (Laid-open No. 42455/1989) (Dainippon Printing Co., Ltd.), March 14, 1989 (14. 03. 89), Claims; drawings (Family: none)	1 - 9

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

December 9, 1997 (09. 12. 97)

Date of mailing of the international search report

December 16, 1997 (16. 12. 97)

Name and mailing address of the ISA/

Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP97/04020

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Microfilm of the specification and drawings annexed to the request of Japanese Utility Model Application No. 156893/1988 (Laid-open No. 77666/1990) (Nissho Corp.), June 14, 1990 (14. 06. 90), Claims; drawings (Family: none)	1 - 9

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